Analysis of Diatom Blooms Using DNA Fingerprints

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LONG-TERM GOALS

My long-term goals are to understand the biological and environmental factors that dictate the timing and magnitude of diatom blooms. I am particularly interested in how species behavior is coupled to environmental conditions and how the extent of genetic and physiological diversity within a population influences its future ability to bloom.

OBJECTIVES

The premise that guides my research is that phytoplankton community dynamics reflect a complicated interaction between environmental variability and the inherent genetic and physiological variation present within individual species of phytoplankton. My goal is to determine just how diverse – at both a genetic and physiological level - individual species of diatoms are and how this diversity is shaped by different environments and on different time scales. Ultimately, this should allow me to determine how population diversity is coupled to future blooming capabilities.

APPROACH

My studies were originally motivated by a small body of literature that suggested that individual isolates from single diatom species could display substantially different physiological characteristics (e.g., Gallagher 1980; Brand 1981). Although extremely provocative, these early studies were labor intensive and by necessity, conclusions were based on limited sample size. To test the potential ramifications of these results - namely, that physiological and presumably genetic variability within diatom populations might explain why bloom dynamics have been so difficult to predict - we needed techniques that would allow us to examine in more detail this presumed coupling between environmental and biological variability. We chose to examine diversity within diatom populations by utilizing a new generation of high-throughput DNA fingerprinting techniques that had traditionally been used to study populations of large, multicellular organisms. Our studies focus on the unicellular centric diatom *Ditylum brightwellii* because of the importance of this diatom in coastal waters and because of the ease of identifying it in mixed populations.

We base our DNA fingerprints on highly repetitive regions of DNA known as microsatellites. These repetitive sequences are generally considered “junk” DNA with no obvious function. The beauty of these “junk” microsatellites for fingerprinting studies is that the length of any given repetitive region can vary dramatically between individuals and so can be used to define individuals. We determine

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14. ABSTRACT
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15. SUBJECT TERMS

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genetic diversity within *D. brightwellii* populations by first isolating individual cells into about 1 ml of media (in a 48-well plate) and then allowing the cells to divide asexually (1-2 weeks) until ~2000 cells per well are present. Each isolate can be thought of as a different individual composed of 2000 genetically identical cells. Subsets of isolates are maintained in culture for physiological studies. DNA is extracted from all isolates (the DNA can be stored for later analysis) and polymerase chain reaction (PCR) is used to amplify specific microsatellites. The length of a microsatellite defines its allele size; the composite of allele sizes at different microsatellite loci defines the DNA fingerprint for an individual. The goal over the past year has been to determine whether we can take this analysis one step further and use composites of individual DNA fingerprints to define populations.

WORK COMPLETED

We have developed and optimized PCR primers for 3 microsatellites in *D. brightwellii* – 2 highly variable loci consisting of dinucleotide repeats and 1 less variable locus consisting of a tetranucleotide repeat (Table 1). The size range and observed number of alleles per locus provide an indication of marker variability within populations and consequently, the usefulness of the marker for distinguishing different individuals; both these parameters will inevitably increase as more individuals are analyzed. A third dinucleotide microsatellite locus is currently being optimized. With only two microsatellites we had the capacity to recognize up to 4,065 different DNA fingerprints (genotypes) within a population (Rynearson and Armbrust 2000). With this newest combination of markers, we can identify as many as 18,478,800 different genotypes, an amazing level of sensitivity.

Table 1. Attributes of currently used microsatellite loci for DNA fingerprinting D. brightwellii cells.

Microsatellite Locus Designation	Repeat Type	Size Range (bp)	Observed Number Alleles
Dbr4	Di-nucleotide	230-328	24
Dbr9	Di-nucleotide	183-369	58
Dbr10	Tetra-nucleotide	189-232	8

Over the past year, acquisition of new equipment (purchased as a result of ONR expansion funding and a DURIP award) has allowed us to dramatically increase sample throughput. For example, we have decreased the amount of diatom biomass needed by 2-3 orders of magnitude, the amount of time needed to extract DNA by 25-fold and the amount of time needed to generate DNA fingerprints by ~20-fold. This increase in throughput translated into a substantial expansion of our field program in Puget Sound, WA. We have now isolated *D. brightwellii* cells from 1) three sites within Hood Canal on a single day in November; 2) two sites within Admiralty Inlet during August, and one of the same sites within Admiralty Inlet during September and October; 3) two sites within Hood Canal, two sites within the Main Basin, and one site within Admiralty Inlet on a monthly basis from February to June; and 4) a single site within Dabob Bay on a daily basis for 11 days during a *D. brightwellii* spring bloom (Figure 1, Table 2). We now have in our collection, DNA isolated from over 1800 *D. brightwellii* isolates; a subset of our isolates have been maintained in culture for varying amounts of time. We are in the process of determining DNA fingerprints for all these samples.

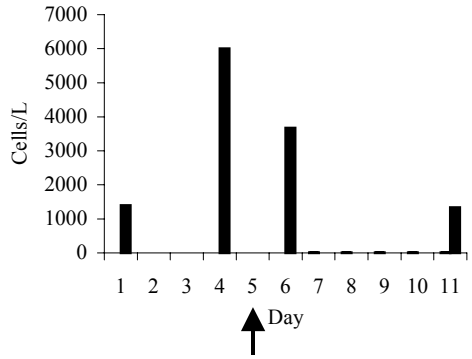

Fig. 1. Change in D. brightwellii cell concentration during a spring bloom in Dabob Bay. Arrow at day 5 indicates timing of a wind event.

Table 2. Summation of successful isolations collected during the spring bloom.

Day	Number Isolates Obtained (number attempted)
1	32 (48)
2	80 (96)
3	72 (96)
4	88 (96)
5	88 (96)
6	64 (96)
7	96 (96)
8	95 (96)
9	48 (96)
10	88 (96)
11	96 (96)
Total	847 (1008)

RESULTS

Over the past year, our focus has been on determining whether we can identify *D. brightwellii* subpopulations with distinct physiological and genetic characteristics. The work has been conducted by myself, my graduate student, Tatiana Rynearson, and an undergraduate, Chuck Lausche.

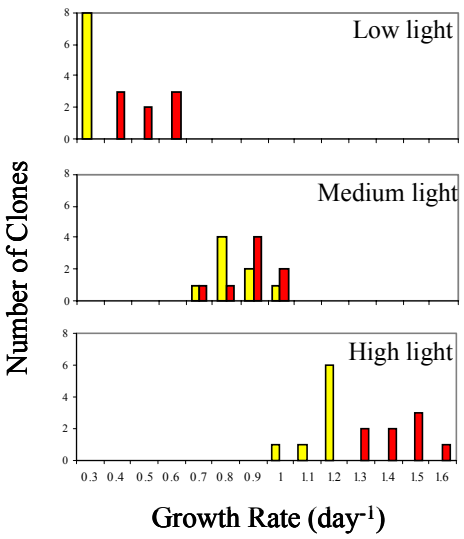

Fig. 2. Maximum growth rates of Hood Canal (yellow) and Admiralty Inlet (red) isolates maintained at three different light intensities.

Our physiological studies have focused on *D. brightwellii* cells from two sites with dramatically different flow regimes – the relatively quiescent waters of Hood Canal and the highly mixed waters of Admiralty Inlet. Currently there is no obvious way to measure *in situ* physiology of individual diatom cells in a high throughput manner. We instead measure the physiological potential of cells in the lab. Subsets of isolates are maintained in exponential growth in nutrient-replete media under different light intensities and maximum growth rates are determined for each isolate under each condition. Remarkably, cells isolated on the same day from the same liter of water in Hood Canal, for example, can differ in their maximum growth rates for a given light intensity by as much as 33%. The greatest difference in growth rates at a given light intensity (low light) was between an isolate from Hood Canal with a maximum growth rate of 0.3 doublings per day and one from Admiralty Inlet that grew more than twice as fast at 0.68 doublings per day. This implies that an enormous range in physiological potential is maintained within populations of *D. brightwellii* found in different parts of

Puget Sound. We have interpreted these physiological data to indicate that small-scale environmental variability, as presumably occurs even in the relatively stable waters of Hood Canal, is critical to maintaining physiological diversity by preventing individual cell lines from dominating a population (Rynearson and Armbrust 2000).

A clear outcome of our growth rate studies was that, in general, Hood Canal isolates grew more slowly than Admiralty Inlet isolates, regardless of which light intensity was tested (Fig. 2). A simple explanation for this result is that the slowly moving waters of Hood Canal allow development of an indigenous population perhaps specifically adapted to this environment. These slowly growing cells are expected to be rapidly out competed in a more mixed environment such as the Main Basin. Thus distinct environments with distinct *D. brightwellii* populations appear to exist within Puget Sound despite the fact that all waters within the Sound eventually mix.

The dramatic difference in growth rates between the Hood Canal and Admiralty Inlet isolates suggested that large-scale environmental forcing generates genetically distinct populations of *D. brightwellii* composed of unique combinations of cells with different genotypes. We have focused on Admiralty Inlet where oceanic waters from the Strait of Juan de Fuca tidally mix with estuarine waters from the Sound. In collaboration with Dr. Jan Newton from the Washington State Department of Ecology (DoE), we sampled *D. brightwellii* at the same site in Admiralty Inlet on August 20 and 21, September 17, and October 23 and at a second site, just a ¼ mile away on August 21. These samples were collected throughout the tidal cycle, but always during Spring tide. We isolated approximately 60 cells each time. Forty to fifty-three isolates grew in the August samples and 48 isolates grew in September, respectively. Only 27 isolates grew in October, perhaps indicating that the population was less healthy then. At each location, water column data - CTD, nutrients, primary productivity – were collected in collaboration with DoE.

The isolates were DNA fingerprinted at three loci and both genotypic and allelic distributions were compared between the different Hood Canal and Admiralty Inlet samples. We used a log-likelihood based exact test and determined that 1) as expected, the populations sampled on August 21 at two locations ¼ mile apart were not significantly different from one another, indicating that this population was cohesive for at least ¼ mile, 2) the two populations sampled at the same site but 1 day apart in August (20, 21) were significantly different from each other ($p < 0.001$) – one sample was taken just after ebb tide (mostly estuarine) and the other just after flood tide (mostly oceanic), 3) both August populations were significantly different from both the September and the October population ($p< 0.001$) at the same site, 4) the September and October populations were not significantly different from one another, and 5) the Hood Canal sample was significantly different from all the Admiralty Inlet samples ($p<0.01$). A simple comparison of allele distributions also illustrates population differences (Fig. 3). For example, the August 20 population was dominated by individuals with Dbr10 allele sizes of 192 bp, but no individuals with allele sizes of 208 bp. In contrast, the September and October populations were dominated by individuals with allele sizes of 208 bp and very few with allele sizes of 192 bp. Similar results were obtained with the other two microsatellite loci (data not shown). These results are extremely encouraging and suggest that we can define populations by genetics. It is particularly intriguing that the September and October populations are indistinguishable from each other. CTD profiles indicate that both samples likely came from water masses with oceanic influence. We do not yet have a ready explanation for why the oceanic population appears to have stabilized from September to October, but it should be noted that this work is the first time anyone has been able to examine diatom populations at this level.

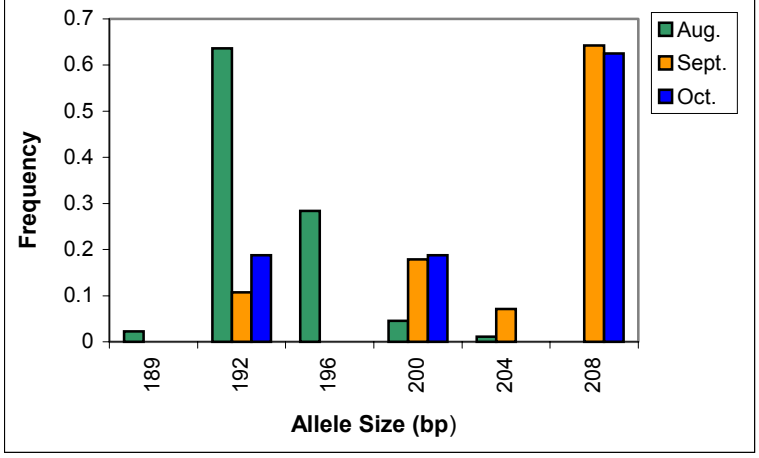

Fig. 3. Dbr10 allele distributions for isolates sampled from Admiralty Inlet on August 20 (green), September (orange), and October (blue).

IMPACT/APPLICATION

Diatom population dynamics dictate to a large extent both the ecology of coastal ecosystems and the optical properties of these waters. And yet, relatively little is understood about which combination of factors dictate the timing, composition, and magnitude of a diatom bloom. We combine physiological studies with sensitive molecular fingerprinting techniques to examine in detail how populations respond to changing environmental conditions. Ultimately, this will allow us to model how environmental, genetic and physiological variability combine to determine the formation and maintenance of blooms.

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Rynearson, T. A., E. V. Armbrust, and J. A. Newton. 2001. Genetic diversity and population structure in the diatom *Ditylum brightwellii* in Admiralty Inlet, Puget Sound. Pacific Estuarine Research Society Annual Meeting. *Tatiana was awarded "best student presentation" for this talk.*

Rynearson, T. A. and E. V. Armbrust. 2001. Genetically distinct populations of the centric diatom *Ditylum brightwellii* identified using microsatellite markers. American Society of Limnology and Oceanography Annual Meeting.